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(54) Devices for use in chemical test procedures.

(57) A specifically-reactive sample-collecting and testing device for use in assaying an analyte, said device possessing a cavity or cavities each having a dimension small enough to enable sample liquid to be drawn into the cavity by capillary action, wherein one surface of the or each cavity carries an immobilised reagent having specific affinity for said analyte and the same or another surface of the or each cavity carries, in releasable form, a further reagent having specific affinity for said analyte, the surface which carries the immobilised reagent being a surface of a transparent solid plate which in use acts as a light-transmissive waveguide and which forms a wall of the or each cavity, said plate having an edge which is substantially optically smooth and transverse, i.e. at some transverse angle but most preferably perpendicular, to the plane of the plate, the immobilised reagent and the further reagent being such that the result of any specific interaction with the analyte is optically measurable.

EP 0 422 708 A2

DEVICES FOR USE IN CHEMICAL TEST PROCEDURES

This invention relates to devices for use in chemical (especially biochemical or clinical) test procedures.

The devices are, in certain embodiments, intended for use in specific binding assay procedures, among which an important group is constituted by immmnoassay procedures. Examples of such immuno assays, especially enzyme-linked immunoassays, are cited in Specifications Nos. EP 0 042 755, GB 2 074 727, GB 2 086 041, and GB 1 548 741.

Previously, immunoassay procedures have often been carried out using so-called microtitre wells, conventionally of about 0.5ml working capacity, amongst a variety of other liquid containers for the assay reaction liquids. Other devices and arrangements for handling immunoassay materials are described in Specifications Nos. EP 0 31 993, GB 1 571 872, GB 1 584 129 and GB 1 414 479, for example.

In particular, the prior art contains numerous disclosures of analytical devices for handling and metering small volumes of test samples.

GB 2 090 659 (Instrumentation Laboratory, Inc.) describes test strips constructed with a self-filling metering channel and a lip or inlet on which a sample of more than about 10 microlitres of for example whole blood can be placed, so that (for example) 10 microlitres is taken up by capillary action to react with a reagent carried on a fibrous pad above a filter layer beneath a transparent window. The result can be viewed by the unaided eye, e.g. as a colour reaction.

GB 2 036 075 (H E Mennier), GB 1 104 774 (J P Gallagher), EP 0 057 110, 0 034 049, 0 010 456 (Kodak), all describe some other aspect of the uses of capillary channel or chamber dimensions for handling biological or test fluids.

GB 1 530 997 (Monsanto) describes the use of coated optical fibres which can be used in tests that change the light transmitting capabilities of the waveguides via reactions of the coatings, e.g. antigen-antibody reactions. WO 81/00912 (Buckles) also describes fibre-optic devices in which the fibre surface or surroundings modify the light transmission through the core.

USP 3 939 350 describes optical measurement of fluorescent material bound to the surface of a solid transparent prism by a method involving a single total internal reflection and interaction of the evanescent wave at the surface of the prism with the bound material.

EP-A-0075353 (Battelle) makes specific reference to the exponentially-decaying (evanescent) external radiation due to light which is propagated longitudinally in a fibre, and its interaction with coatings, and this principle is also taken up in the immuunoassay test devices of EP-A-0103426 (Block) in which light of fluorescence excitation as well as emission wavelengths is propagated within an antigen- or antibody-coated optical fibre or plate contacting a capillary-dimensional sample liquid volume bounded by a tube or another plate and containing a fluorescent-tagged binding partner of the material coated on the fibre or plate.

According to the invention to be described here, capillary fill cell devices,which can be conveniently manufactured, are provided to facilitate in particular specific binding assays using very small liquid samples.

According to the invention we provide a specifically-reactive sample-collecting and testing device for use in assaying an analyte, said device possessing a cavity or cavities each having a dimension small enough to enable sample liquid to be drawn into the cavity by capillary action, wherein one surface of the or each cavity carries an immobilised reagent having specific affinity for said analyte and the same or another surface of the or each cavity carries, in releasable form, a further reagent having specific affinity for said analyte, the surface which carries the immobilised reagent being a surface of a transparent solid plate which in use acts as a light-transmissive waveguide and which forms a wall of the or each cavity, said plate having an edge which is substantially optically smooth and transverse, i.e. at some transverse angle but most preferably perpendicular, to the plane of the plate, the immobilised reagent and the further reagent being such that the result of any specific interaction with the analyte is optically measurable.

The devices enable convenient sample collection and carrying out optical analysis in situ of the products of reaction of the sample with reagent(s) contained in the devices. The waveguide plate can be transparent to infrared,visible, and/or ultraviolet light, and one method of using the devices is to arrange for a fluorescent material to become bound to the immubilised reagent to a variable extent depending on the assay and the sample material of interest, and then to carry out optical measurement of the resulting bound fluorescent material, for example as described in EP-A-170376.

The immobilised reagent in the device can for example be an immobilised antigen or antibody which is capable of binding a fluorescent or luminescent or coloured component of an assay mixture. But it is understood that any immobilised material can be used that specifically interacts with another component of a test reaction material in a way that gives optically measurable results, depending only on the kind of test in question.

The surface of the cavity opposite to the reagent-bearing waveguide surface can, as in examples illustrated below, be formed of a second similar transparent plate. However, this is not necessary, and for some forms of test it may be suitable to use a light-absorbing or opaque or reflective opposite wall to the capillary cavity.

In some useful examples of the devices of this invention, a selective barrier such as a filter or dialysis membrane can be fitted to ensure that the takeup of sample liquid into the capillary-dimension cavity is selective to exclude unwanted material. Such unwanted material will depend on the nature of the test but may include cells such as blood cells, cell debris or dispersed high-molecular-weight material A suitable filter can be for example a paper filter located at the sample entrance to the capillary cavity or a strip or area of filter or dialysis membrane material fixed at or in the capillary cavity.

It is also within the scope of the invention that the area covered by an immobilised reagent within the capillary cavity, e.g. an immobilised antigen or antibody, can be significantly less than the whole area of the capillary cavity, and arranged so that all of the sample liquid must pass over the immobilised reagent as liquid is drawn into the cavity. In this way a sample-concentration effect can be achieved, with a resulting surface concentration of relevant analyte material (e.g. complementary antibody or antigen) higher than would result from uniform adsorption over the whole capillary cell area. Such an immobilised coating over a restricted threshold area of the capillary cell surface can form in effect a selective barrier to allow passage of sample liquid beyond the barrier with selective retention of an analyte material of interest.

In the use of devices according to the invention, a drop of sample liquid can be placed on a collection surface of the test device, or else the device can be dipped into a quantity of liquid to be sampled. The further reagent having specific affinity for the sample analyte (which reagent will for convenience be referred to hereafter as an ancillary reagent) is carried on a part of the device to be contacted in use by sample liquid, e.g. on a surface of the capillary cavity or a surface of a filter if present. More than one ancillary reagent may of course be present, if desired.

Further variants and particular features of the test devices are described for example below.

The devices of the present invention may, for example, be manufactured according to the method described in EP-A-171148.

The ancillary reagent(s) are in the form of a releasable reagent coating, e.g. a coating of releasable antigen or antibody, or derivative thereof, for example held in a solid humectant, e.g. sucrose glaze, with specificity appropriate to a desired assay. The immobilised specific binding material may be for example a covalently bound antigen or antibody or derivative thereof, also possibly coated with a humectant, to form an immunosorbent, with specificity appropriate to a desired assay.

In certain examples of devices according to the invention, the cavity of the device can be a thin planar cavity between two opposite walls forming a cell, and preferably cemented or made into an integral unit. In some cases, for example, the device can include a bonded structure of transparent plates similar to the structure of an unfilled liquid crystal display device as obtained as an intermediate stage in manufacture of liquid crystal displays.

Thus devices according to the invention include those possessing a (for example disposable) translucent or transparent capillary cell, which can be made by the methods described herein, for carrying out specific binding assays, comprising a pair of opposite transparent plates spaced less than about 1mm apart, and sealed together to form an apertured liquid-retentive cell able to take up and retain by capillary action a (preferably defined) volume of (usually aqueous) liquid, and carrying on at least one of its internal surfaces a coating of immobilised specific binding agent with a specificity appropriate to the assay to be carried out. "Defined volume" means a volume that is determined substantially by the shape and configuration of the cell itself and not appreciably by the volume of sample if applied in excess.

It is important to have an accurately defined (parallel) cavity of capillary gap dimensions in the devices of this invention, but not so important that the total volume taken up by the capillary gap should be defined: the important parameter is rather the volume dosing per unit area of the optical reagent bearing surface of the waveguide, and this is achieved by an accurately defined parallel spacing of the opposite walls of the cavity in the device. The spacing is preferably within the broad range about 0.01 to 1mm, e.g. of the order of 0.1mm, e.g. 0.03-0.3 mm, as a compromise between, on the one hand, too wide spacings leading to excessive diffusion time of materials in the sample across the gap to the reagent-bearing surface and, on the other hand, too narrow spacings which may collect too little sample.

Materials suitable to form the cavity are for example glass, eg. sheet soda glass about 1mm thick, silicas and plastics sheet material, eg. acrylic plastics material.

Where plastics materials are used to form the capillary cells, they can for example be used in the form of precision mouldings, e.g. provided with spacers such as ridges to achieve controlled spacing of the component walls of the capillary cell cavities.

In some cases the cell has an outer surface portion or lip to which a quantity of sample sufficient to fill the cell can be applied and from which it can easily be made to run into the capillary cell by capillary action. Such a lip can easily be formed by an extension of one of the plates, outwardly beyond the cell aperture, for a distance sufficient to give a surface area large enough for convenient sample loading. It can also if desired be given a liquid-conductive shape, such as a groove or channel leading towards the capillary cell inlet. An alternative form of inlet is one formed by an aperture in one wall of the capillary cell, e.g. a hole that exposes an area of opposite wall of the cell on to which a sample can be loaded. Selective barriers such as those described elsewhere herein, such as filters and dialysis membranes, can also be located in or adjacent to such inlet apertures, and may be pre-dosed with dry releasable reagents. It is especially convenient to incorporate these features in the plastics capillary cells, and in such cases precision-moulded apertures supplied in a moulded plastics sheet can provide the perpendicular optically flat ends of the capillary cells when the resulting assembly of plural capillary cells is separated into individual cell units during a preferred method of manufacture.

Preferably the sealing of the cell can be achieved by using a line of epoxy resin, leaving an aperture, e.g. extending the resin along two opposite sides of a rectangular capillary cell, to give a filling aperture and a further aperture left to allow the exit of air from the capillary cell as it fills up. Suitably, the resin can comprise solid particles to ensure a desired spacing for the plates as they bed down on the resin. Particles such as substantially monodisperse ballotini (fine glass particles) of diameter about 100 micron or otherwise corresponding to the chosen capillary gap, or short glass fibre lengths of for example 8 micron diameter and 50-100 micron long (e.g. made by mortar-grinding of long glass fibre and exclusion of long residual fibres by sieving), are suitable to regulate small spacings of the order of the diameter of the ballotini or the fibres. Generally, spacings in the range 5 to 500 microns can be chosen, by way of non-limitative example. Fibres are preferred for very narrow gaps, as they are more easily obtainable in diameters less than about 50 micron than are monodisperse ballotini: ballotini are preferred for the wider gaps.

The specific binding agent to be immobilised on one surface of the cavity can be chosen among any of those normally used for purposes of specific binding assay, especially antigens and antibodies. Suitable examples are antiglobulin antibody, or antibody specific to human apolipoprotein A_1 or B, or anti-steroid antibody, eg. specific for oestrone-3-glucuronide or pregnanediol glucuronide, or non-immunological binding agents such as concanavalin A, or cibacrom blue. They can be immobilised to the glass or silica or plastics surface in any of the ways otherwise practised for such immobilisation. For example, it can be useful simply to coat and dry the binding agent and sucrose on to the carrier surface, either simultaneously or successively. Covalent and other immobilisation can be achieved where desired in any of the ways mentioned in EP specification 0 014 530 (Unilever), and references cited therein, especially in the case of plastics materials, and in any of the ways mentioned in "Immobilised Enzymes for Industrial Reactors" (ed. Messing, Academic Press, 1975; especially Filbert, chap 3), or in for example USP 3 652 761 or GB 1 530 997, for a wide variety of carrier materials including siliceous materials such as glass and silica.

The format of immunoassays, especially e.g. fluoroimmunoassays, which can be carried out in these capillary cell devices corresponds to the known formats of other immunoassays of the prior art. For example, a sandwich test or antiglobulin test can be carried out in which an immunosorbent and a fluorescent ligand (e.g. both antibodies, or in the antiglobulin test respectively an antigen and antiglobulin) both have specific affinity for the analyte (the antigen or antibody) under test.

The analyte can be contacted with the immunoadsorbent in the absence of the fluorescent ligand and this reaction can be followed by contact between the treated immunoadsorbent and the fluorescent ligand released in slow or delayed manner from a releasable coating.

Similarly, luminescent assays may be carried out using labels which participate in chemiluminescent or bioluminescent reactions, e.g. luciferase or horseradish peroxidase.

Embodiments of the invention are illustrated for example by the accompanying Figures 1-7 and associated description.

Figure 1 shows a diagrammatic section through a disposable capillary cell device according to one embodiment of the invention.

Figure 2 shows a diagrammatic plan of the cell device of Figure 1, and includes a line I-I to show the line of section of figure 1.

Figure 3 shows in schematic perspective view a specifically-reactive capillary cell device according to an embodiment of invention.

Figures 4 and 5 show a schematic plan and section of the device of Figure 3.

Figures 6 and 7 show a corresponding schematic plan and fragmentary section of an alternative device, a modification of the device of Figures 3-5.

Figures 1-2 show a capillary cell device of a size to be handled easily, e.g. about 3cm x 1.5 cm. The

device comprises upper transparent (e.g. plastics, glass, or silica) plate 1 and lower transparent (e.g. similar) plate 2 (about 1mm thick) fixed together in parallel opposed and spaced relation, less than 1mm apart, by bonding tracks 3 of suitable (e.g. epoxy) adhesive to form a capillary cell cavity 4, open at both ends, which communicates with the outside through a first discontinuity in the bonding 3 arranged to form a cell aperture at side 5 of plate 1. Another discontinuity is present at the other end of bonding 3, to leave another aperture, to allow exit of air when a sample liquid is loaded into the cell. Plate 2 is larger than plate 1 and has a portion 6 extending away from the aperture. Portion 6 of plate 2 acts as a platform or threshold or lip onto which a drop of sample liquid can be applied, so that this liquid can be made to fill the capillary cell cavity 4 by capillary flow. Cavity 4 attracts and contains a definite and adequately reproducible volume of liquid when loaded in this way.

Immobilised to the inner surface of the capillary cell is a layer 7 of material relevant to the test procedure in which the capillary cell is to be used. In the example shown in the drawings the layer 7 is a patch of material carried on plate 2. For the purpose of immunoassay, it can be for example an area of immobilised immunosorbent sensitisation, e.g. immobilised antibody, relevant to an immunoassay. There can be more than one such layer, e.g. a layer on plate 1 as well as plate 2, or a superimposition and/or side-by-side plurality of layers on either plate. For similar or other purposes, the layer 7 or other layer(s) lining the internal surface(s) of the capillary cell can include an electrically conductive layer or layers, as described in EP-A-170375 and in such a case conductive external connections can be provided by means of conductive tracks or connectors from the interior of the cell to the exterior of the cell, if desired, passing between bonding layer 3 and the surface of the plates. These can for example be made in a manner known per se and used in the conventional surface fabrication of conductive tracks as often employed in the manufacture of semiconductors and liquid crystal displays, e.g. as referenced below.

On the same or another inner surface of the capillary cell as layer 7 is carried a layer 10 of ancillary reagent. In the example shown in the drawings the layer 10 is a patch of material opposite layer 7 and carried on plate 1.

When the cell is intended for making optical measurements, either plate 1 or plate 2 or both should be transparent or translucent.

The section shown as Figure 1 presents plates 1 and 2 spaced apart because the line of section does not extend through the bonding tracks 3.

The fabrication of a plurality of cells such as that of Figures 1-2 is described in detail in EP-A-171148 (see, in particular, Figures 3 and 3a and associated description).

The capillary cell device of Figures 1-2 can if desired be provided with any convenient form of handling-piece or holder and for this purpose may be provided with any convenient form of fixed or releasable connexion arrangement to engage with such a holder where this is not formed in one piece with the cell device.

For convenience of optical measurement, it is desirable that the edge or edges of plate 1 and/or 2 which is/are optically smooth is/are perpendicular to the plane of the plate. The remaining edges may if desired be coated with black paint or other light absorbing material. Alternatively or additionally, absorbing pigment such as carbon black may be incorporated in the cement such as epoxy that is used to bond the two plates together.

In use, the device of Figures 1 and 2 can transmit fluorescence from an adsorbed fluorescent material along either or both of the plates 1 and 2 in the manner of a waveguide or optical fibre. Transfer of useful light across the boundary of the plate can occur by the evanescent wave located very close to the interface. If desired, a thin layer of for example silica or magnesium fluoride, of the order of a quarter-wavelength thick at the wavelength of interest, may be fabricated on to the plate before deposition of the biochemical reagent, in order to improve light transfer across the boundary. The thickness and refractive index of the dielectric layer are preferably together chosen to maximise the intensity of the evanescent wave associated with light transmitted in the plate in a path that corresponds to total internal reflection near (and slightly above) the critical angle for the boundary between the plate material and the sample liquid, e.g. usually in the range about 1°-5° above the critical angle. The optimum refractive index of the dielectric layer is as close as possible to that of the sample liquid: practically it is most usual to choose a dielectric of magnesium fluoride ($n = 1.38$) or silica ($n = 1.46$) when glass is used. The optimum thickness for the layer is expressed in terms of the angle of incidence P of the light in the plate at the plate-dielectric interface, and the refractive indices of the media: n_1 of the sample liquid, n_2 of the plate, n_3 of the dielectric, L is the wavelength of the light in use:-

$$t = {}^{L}/_{4} \cdot \frac{\left[1 - {}^{2}/_{\pi} \arccos \sqrt{\left(\frac{n_3{}^2 - n_2{}^2 \sin^2 P}{n_3{}^2 - n_1{}^2}\right)}\right]}{\sqrt{\left(n_3{}^2 - n_2{}^2 \sin^2 P\right)}} .$$

In further derived alternative examples, continuous or discontinuous thin metal layers e.g. of silver or indium, preferably overlain by a vacuum-deposited very thin corrosion-resisting layer such as silica, may also be deposited on either or both plates to enable the device to be used in corresponding other methods of optical analysis known in themselves, which exploit such layers, e.g. the per se known phenomenon of surface plasmon resonance, as described in B.Liedberg et al , Sensors and Actuators, 4 (1983) 299-304. In any of these cases the immobilised layers of biochemical reagents are supplemented by releasable ancillary reagent coatings, e.g. formed by air-drying protein-sucrose mixtures in thin films on the plates: selected and combined according to the particular test chemistry to be performed in the device. The range of chemical or binding reactions that can form part of the tests to be carried out spans the range of known binding-reaction tests, and includes enzyme-linked, fluorescence, luminescence, binding and quenching reactions of any kind based on solid-phase immunoadsorbent or other specific binding adsorbent.

Further details of procedures and material to immobilise reagent(s) on the desired plate of the capillary cell device of Figures 1-2 are now given. The immobilisation is conveniently carried out on a large sheet to form the said plate of a multiplicity of the devices.

A sheet of (e.g. soda) glass for example about 1mm thick, and large enough to contain a 2-dimensional array of cell areas, with a plurality of several cell units in each direction, is cleaned by any suitable method, eg. by detergent and ultrasonic treatment and if need be by solvent vapour degreasing in known manner, or by successive hot (80°C) treatments with ammonia hydrogen peroxide and hydrochloric acid/hydrogen peroxide, water-rinsing and airdrying, e.g. at 115°C for 30 minutes. A pattern of patches of a desired protein or other coating is then applied by the following or equivalent technique. Covalent coupling of antigen or antibody or other protein is achieved by first reacting the glass with a silane-based coupling compound in known manner. A suitable such reagent is for example, as used here, a terminal amino-alkyl trimethoxysilane, e.g. the 3-aminopropyl compound, used at a concentration of suitably about 2% v/v in acetone. In alternative methods, another reagent substantially as described in US Patent 3 652 761 can be used instead. After reaction with the amino-silane reagent, the amino terminals immobilised on to the glass are in turn reacted with (e.g. 2% pH 7) glutaraldehyde, excess reagents are removed and the activated glass with immobilised aldehyde groups is reacted with the protein in solution (e.g. 1 mg/ml antibody immunoglobulin), according to component techniques well known in themselves. Other proteins can be applied at choice in concentrations of the order of 0.1-1 mg/ml. Treatment at about pH 9.5 for 2 hours at 37°C has been found suitable here. A suitable final active protein loading rate on the glass surface can be for example about 0.5 microgram/cm^2. This is thought to constitute a continuous or near-continuous layer. The dosage or density or specific activity of the immobilised layer is determined by the sensitivity requirements of the particular assay chemistry, which in itself forms no part of this invention. Excess reagents can be removed by for example washing in strong buffer (0.1M acetate, 0.5M NaCl, pH4-5), then neutral buffer washing, (pH 7-7.4), followed by pH 9-10 washing and neutralisation, e.g. with neutral tris buffer.

An alternative and sometimes preferred method for coupling protein to glass employs an epoxy-silane reagent, especially the glycidyloxypropyltrimethoxysilane (e.g. 2% v/v in toluene, 70°C for 2½ hours), according to DP Herman et al., J.Chromatogr.Sci., (1981), 19 (9) 470-6. With this reagent the use of aldehyde reagents can be omitted, as the epoxysilylated glass can react directly with protein.

It is usually desirable to apply a stabilising coating to such layer-coated surfaces such as a coating of a solid humectant such as sucrose. A suitable example of such a coating is an 8 micron-thick solid sucrose coating applied for example by spin-coating the plate with sucrose solution and drying in air.

Releasable coatings of ancillary reagent can be applied with per se known composition, e.g. carried in admixture with a solid humectant, such as sucrose glaze. We find that it can be desirable to include detergent or inert protein in such coatings (or a soluble salt or buffer material) especially where the active material to be released is itself a protein, and it can be especially desirable to avoid large excess of the reagents in such releasable coatings in relation to the test reactions in which they are to take part.

Uniformity of the coating processes can be important, and if appropriate can be checked by test procedures in which radiolabelled and/or fluorescent protein is coated, and then optionally reacted with a

further and preferably differently-labelled binding agent. Uniformity of the coating and of its binding capacity can then be checked by surface fluorescent measurement and/or surface radioactivity measurement, eg. using a laboratory gamma-ray scanner.

If it is afterwards desired to etch the coating on the glass, the coated glass is then placed in a confined atmosphere substantially free of moisture or air draughts, e.g. it can be brought close to another flat inert surface to reduce the air gap on the coated side to about 1mm or less. The glass is then illuminated with an ultraviolet patterned image (using preferably light of as narrow as practicable a waveband around 280nm) in a pattern corresponding to areas from which the coating is to be etched away, e.g. a grid pattern, to leave a pattern of surviving protein patches. Illumination can for example be carried out using a GE 7-watt mercury lamp spaced a few centimetres from the plate, for a period of about 5-20 minutes. The illumination pattern can be produced by masking close to the plate, or by a real imaging system. The ultraviolet etching used here relies on the same principle as the u.v. etching process described by J. A. Panitz,and I.Giaver, in Surface Science, 97 (1980) pp 25-42.

Then a u.v.-curable epoxy adhesive is painted on to the coated, eg. patch-coated, glass plate in a desired pattern for forming a connexion with an upper spaced plate. The epoxy adhesive is applied by a silk-screen technique which is conventional in itself, and in itself forms no part of this invention.

The epoxy resin can have a small content of short-length glass fibre, about 20 micron in diameter and about 100-200 micron long, (made for example by grinding long glass fibre in a mortar and sieving to remove residual long fibres). A preferred alternative to the glass-fibre pieces is a content of ballotini in the epoxy resin, used as follows. In order to produce a gap of for example 100 micron, correspondingly-sized ballotini are incorporated in the epoxy: a layer of epoxy a little thicker than the desired spacing between the plates, e.g. 10% thicker, e.g. about 110 micron for a desired spacing of 100 micron, can be laid down by screen-printing, and the addition plate pressed gently into position to spread the epoxy slightly.

If desired, a mirror-image of the first pattern of epoxy adhesive can be applied as a pattern to a second similar sheet of glass, either coated patchwise with the same or a different protein or other coating material, or otherwise uncoated, and the two sheets then brought together, subjected to vacuum or deoxygenation if needed for curing, and cured by ultraviolet illumination. The ultraviolet is applied as an image with a pattern that avoids the patches of coated protein or other material which are to be retained in active form.

After adhesive curing, the two glass plates can be scribed and broken down into individual cell units in any convenient known manner as used in stages in the manufacture of liquid crystal devices, and in particular by the methods referred to in Specifications Nos. CH-627 559, and 629 002, concerning fabrication of liquid crystal display devices.

A convenient form of cell obtainable by this process comprises two substantially parallel opposed layers of glass, air-spaced by about 5-500 micron, which, together with an incomplete frame of bonding material located between them, (having at least one opening for the inward passage of liquid and possibly also the outward passage of air), form a capillary cell able to take up a defined volume of aqueous liquid. Preferably one of the glass layers extends out beyond the opening of the cell to enable a drop of liquid to be placed on its surface and pass either wholly or partly into the cell.

Figure 3 shows in schematic perspective a further example of a specifically-reactive capillary cell device according to an embodiment of the invention. The device shown is a disposable single-use test device for carrying out microchemical testing on very small liquid samples. Figure 4 (not to scale) shows a corresponding plan view and Figure 5 a schematic section along lines 6′-6′ in Figure 4. The device of Figures 3-5 comprises an upper glass plate 1 and a lower glass plate 2 related as plates 1 and 2 of Figure 1. A reactive layer as 7 in Figure 1 is present on the surface of plate 2 but not shown in the drawings in Figures 3-5. An auxiliary reagent (not shown) can be provided as a releasable coating on the opposite wall of the cell, i.e. the surface of plate 1, so that the reagent dissolves in the sample liquid drawn into the cell. The device of Figures 3-5 can be handled by the user by a handlepiece 42 of a supporting and handling frame indicated generally 41 and made of any convenient plastics moulded material. Arms 43 serve to support the cell assembly and locate it in relation to the operative parts of an optical instrument for optical analysis of the contents of the sample cell. End 44 of plate 2 is optically clear, flat and perpendicular to the plane of plate 2, to allow exit of light arising from the contents of the capillary cell between plates 1 and 2. The other edges of plates 1 and 2 are in this embodiment usefully painted black, and epoxy bonding tracks 3, corresponding to tracks 3 in Figure 1, besides their content of 100 micron ballotini to space plates 1 and 2 apart, also contain carbon black granules to help minimise stray light. A filter paper rectangle 45 is located on a part 46 of plate 2 that extends beyond the length of plate 1 to form a sample-receiving inlet: the adjacent open end 47 of the capillary gap between plates 1 and 2 is an inlet for the capillary cell delimited by plates 1 and 2 and bonding tracks 3, and filter 45, of a grade sufficiently fine to exclude the passage of red blood cells, is in contact with open inlet end 47 and preferably slightly underlaps plate 2 at

end 47. Filter 45 is retained in place if desired by adhesive along parallel lines continuing tracks 3, and can if desired be impregnated with a releasable/auxiliary reagent such as for example a pH buffer salt. Portion 47 of plate 2 extending beyond plate 1 to the optical end 44 of plate 2 can if desired be given a hydrophobic coating.

In use, a sample of liquid to be tested, e.g. a drop of whole blood, can be applied to the inlet zone formed by filter 45, and from such a drop a quantity of relatively cell-free liquid is drawn into the capillary cell. Here take place the binding reactions appropriate to the nature of the required test and of the corresponding reagents (not shown) previously carried in prepared dry form on one or both of the opposite internal cell walls formed by plates 1 and 2, and the loaded cell can then for example be put into a photometric instrument, e.g. that described in EP-A-170376 for optical measurement as described therein.

Figures 6 and 7 illustrate a modification of the device of Figures 3-5 The device has two snap-on handle-and-support pieces, one shown at 41, corresponding to 41 in Figures 46 except that it is detachable, and another handle and support piece 71 comprising handle 72, which is a removable snap-on fit over lateral ribs 73 at the optical end of the device, which comprises similar optical end-faces 44 and 74 of both the spaced-apart plates 1 and 2. Handle 42 is a firmer snap-on fit than handle 72.

The device of Figures 6 and 7 is supplied with handle 72 fitted and handle 42 separated. In this condition a cellulose nitrate or acetate microfine filter or dialysis membrane 75 carrying an ancillary reagent (not shown) is exposed at the inlet end of a capillary cell defined as before between plates 1 and 2 and binding tracks 3, but defined here also by a further transverse blackened epoxy bonding track 3a that limits the forward spread of liquid towards ends 44 and 74 of plates 1 and 2, and results in the forward parts of plates 1 and 2 being able to constitute waveguide portions bounded by air on each side. Two lateral gaps are left between tracks 3 and 3a and two corresponding apertures 76 in frame 73 allow for exit of air when the capillary cell fills with sample liquid. Thus, in use, the device can be held by handle 72 and dipped into a source of sample liquid to allow a sample to fill the capillary cell through filter 75. In this embodiment each of plates 1 and 2 carries an immobilised reactive layer 7 and 77 such as an appropriate antibody layer fabricated as described above. If desired, the extent of reactive layers 7 and 77 can be limited to the area shown hatched in Figure 6 on each of plates 1 and 2 between filter 75 and line 78. With such an arrangement, the gently inflowing sample liquid causes release of the ancillary reagent and then passes over the reactive layers which are each able to abstract their respective ligand.

When the sample has been loaded, separate handle 42 can be snapped-on into place and the more weakly-attached handle 72 can be removed, thereby exposing the optical ends 44 and 74 of plates 1 and 2 for measurement as before, with the modification that two different optical properties may be measured at once by virtue of the separate optical waveguides 1 and 2 and different reactive layers 7 and 77 of the device. A corresponding optical measuring instrument therefore locates a diaphragm in a position shown by dotted line 79 to separate the respective lights emerging from plates 1 and 2.

Many of the devices according to the above description can be applied to carry test samples which are to be measured optically by the photometric arrangements and methods described in EP-A-170376.

Claims

1. A specifically-reactive sample-collecting and testing device for use in assaying an analyte, said device possessing a cavity or cavities each having a dimension small enough to enable sample liquid to be drawn into the cavity by capillary action, wherein one surface of the or each cavity carries an immobilised reagent having specific affinity for said analyte and the same or another surface of the or each cavity carries, in releasable form, a further reagent having specific affinity for said analyte, the surface which carries the immobilised reagent being a surface of a transparent solid plate which in use acts as a light-transmissive waveguide and which forms a wall of the or each cavity, said plate having an edge which is substantially optically smooth and transverse to the plane of the plate, the immobilised reagent and the further reagent being such that the result of any specific interaction with the analyte is optically measurable.

2. A device according claim 1, wherein the further reagent is fluorescent.

3. A device according to claim 1 or claim 2, wherein remaining edges of the waveguide are coated with light-absorbing material.

4. A device according to any of claims 1 to 3, wherein interposed between the surface of the waveguide and the immobilised reagent is a thin layer of material to enhance transfer of light across the boundary of the waveguide by the evanescent wave located close to the boundary.

5. A device according to claim 4, wherein the said thin layer of material comprises magnesium fluoride or silica.

6. A device according to any of claims 1 to 3, wherein interposed between the surface of the waveguide and the immobilised reagent is a thin coating of conductive material capable of showing a surface plasmon resonance effect.

7. A device according to claim 6, wherein the thin coating of conductive material is a coating of silver up to about 50 nm thick.

8. A device according to any of claims 1 to 7, wherein the immobilised reagent is selected from an antigen, an antibody or a derivative thereof.

9. A device according to claim 8, wherein the antigen or antibody or derivative thereof is covalently bound to the surface of the transparent sheet material acting as a light transmissive waveguide.

10. A device according to any of claims 1 to 9, wherein the transparent solid plate comprises glass or plastics material.

11. A device according to any of claims 1 to 10, comprising a bonded structure of transparent plates cemented in spaced relationship to leave a thin planar cavity of capillary dimensions between them.

12. A device according to claim 11 wherein one of said plates extends longitudinally beyond the other so as to provide a platform from which sample liquid may be taken up into the capillary cavity.

13. A device according to any of claims 1 to 12 further comprising a handling-piece or holder.

14. A device according to any of claims 1 to 13 further comprising a selective barrier such as a filter or dialysis membrane, or a means for sample concentration, located in, or in the inlet path to, the capillary cavity.

15. A modification of a device as claimed in claim 14 wherein instead of being carried in releasable form on a surface of the or each cavity, the said further reagent is releasably retained in contact with the selective barrier.

16. A device as claimed in any one of claims 1 to 14 further comprising an electrode structure for making electrical measurements, in use, on the contents of the capillary cavity.

17. A sample-collecting and testing device for use in a luminescent immunoassay of an analyte, said device comprising a planar capillary all for the collection and retention of sample liquid, said capillary cell comprising a pair of flat plates fixed together in substantially parallel relationship and sealed along two opposite sides so as to provide fixed opposed inner surfaces defining a capillary cavity with a first aperture at one end thereof to allow uptake of sample liquid in the capillary and a second aperture at the other end thereof to allow exit of air from the capillary cavity as it fills with sample liquid, wherein at least one of said plates is a light-transmissive waveguide having an optically smooth edge which is transverse to the plane of the waveguide and perpendicular to the sealed sides thereof, said waveguide having bound to at least a portion of its inner surface, so as to be contacted in use by sample liquid within the capillary cavity, an immobilised reagent capable of binding, either directly or indirectly, a luminescent-labelled ligand, wherein said immobilised reagent and said luminescent-labelled ligand are appropriate to the test for analyte to be carried out in the device, and wherein said luminescent-labelled ligand is releasably retained within said device such that in use it is contacted by and released into the sample liquid collected therewithin.

Fig. 1.

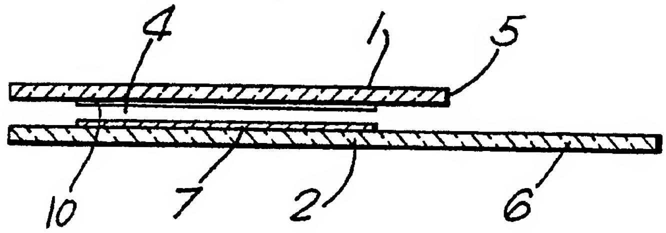

Fig. 2.

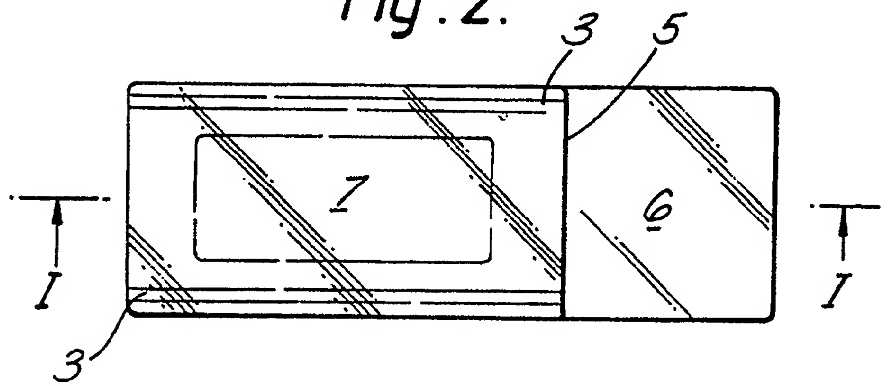

Fig. 3.

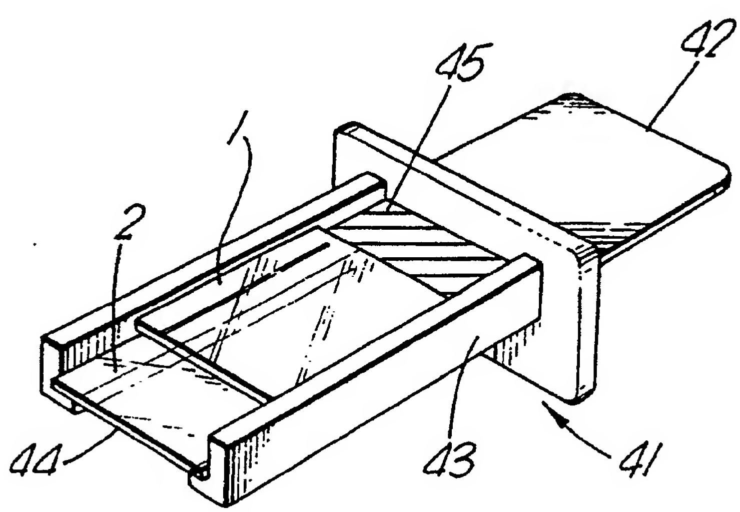

Fig. 4.

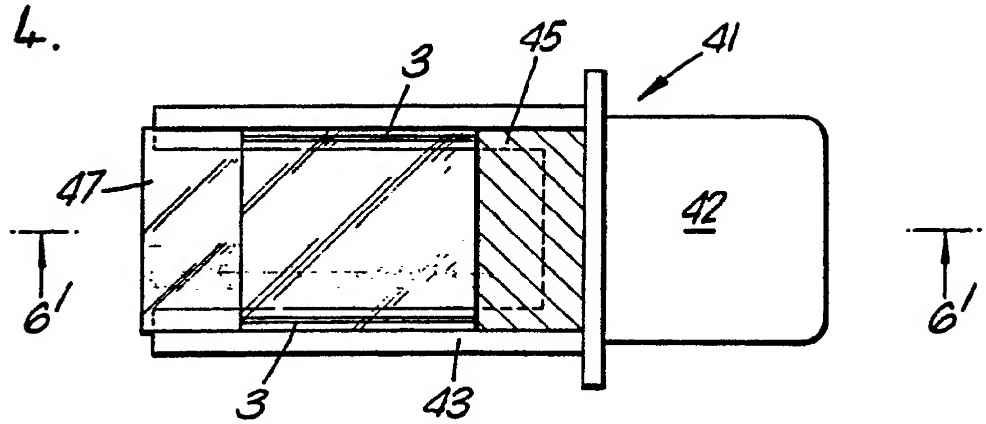

Fig. 5.

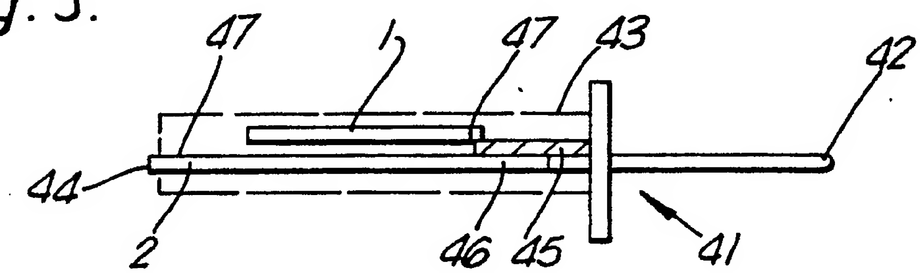

Fig. 6.

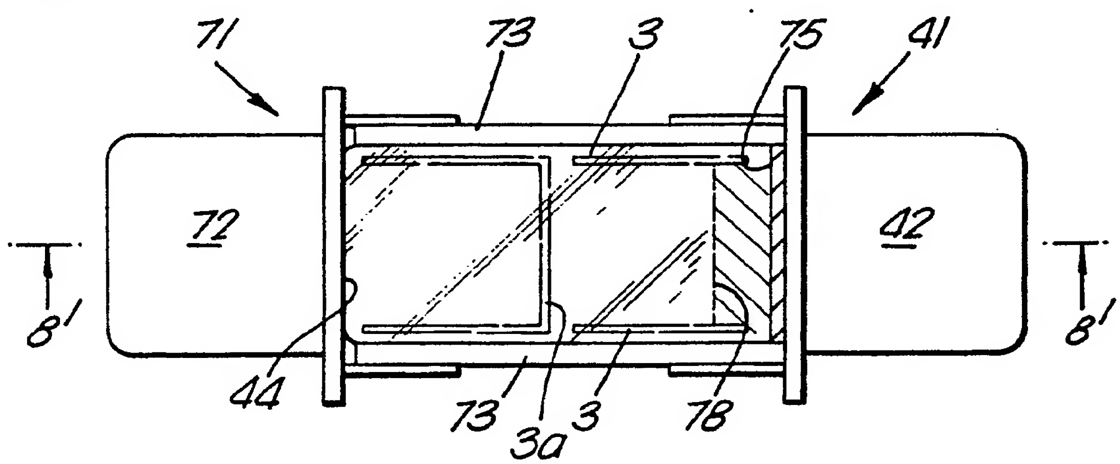

Fig. 7.